HEPATIC METABOLISM OF PHENYLALANINE DURING DEVELOPMENT *

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The phenomenon of substrate induced enzyme formation has been amply documented in studies of microbial systems (1), and similar adaptive changes in enzymic activity in the tissues of vertebrate forms have been described by Knox, Mehler and Lin (2, 3) and by others (4, 5). The suggestion has been made (4, 6) that substrate induction may be a mechanism involved in those changes in enzymatic activities that normally occur during development and differentiation. An extension of this concept might predict that changes in the tissue level of a given substrate during development would be associated with alterations in the activities of enzymes concerned in the metabolism of that substrate. The present studies were initiated to test this hypothesis, using hepatic metabolism of the essential amino acid phenylalanine as a model system. Aside from incorporation into protein, which is common to all amino acids, the known reactions of phenylalanine in mammalian liver include only transamination to yield phenylpyruvate (7–9) and hydroxylation at the *para* position to form tyrosine (10). A previous report from this laboratory (11) established that the enzyme system forming tyrosine from phenylalanine is essentially absent during fetal life in the rat, developing to the level of the adult several days after birth. In the present report, this observation has been extended to other mammalian species and the nature of the enzymic deficiency in the liver of fetal animals has been determined. In addition, transamination of phenylalanine in rat liver has been studied, with particular reference to changes in activity of the enzyme during fetal and neonatal periods of development. A preliminary report concerning some of these studies has been published (12).

METHODS AND MATERIALS

Tissue sources. The rats (Long-Evans) and rabbits used in these experiments were from laboratory colonies. The procedure for the determination of gestational age of rat fetuses has been described (13); the age of rabbit fetuses is estimated from the date of placing the male with the female. Pig fetuses 19 to 20 cm. long (crown to base of tail) were obtained at the abattoir. The sample of liver from a human premature infant was obtained at autopsy, two to three hours after death.

Enzyme preparations. Livers were removed and placed in cold saline or sucrose solutions as quickly as possible. Homogenates were prepared in 0.15 M KCl or 0.25 M sucrose using a Teflon homogenizer; livers of fetal and newborn (less than 10 days of age) litter mates were pooled. Preparation of mitochondria and other subcellular fractions from sucrose homogenates was carried out as described by Schneider (14) with some modifications (15). Fractions I and II, comprising the phenylalanine hydroxylase of rat liver, were prepared essentially as described by Mitoma (16).

Enzyme assays. Phenylalanine hydroxylase activity was assayed by measuring tyrosine formation, using the colorimetric procedure of Udenfriend and Cooper (17); details of additions to reaction mixtures are given in the appropriate tables. The high "blank" content of tyrosine in crude preparations was routinely corrected for by inclusion of samples in which enzymic activity had been initially terminated by trichloroacetic acid. Phenylalanine transamination was routinely assayed in the direction of phenylalanine formation by following the disappearance of phenylpyruvate, using a method suggested by Gilvarg (18). Small aliquots of trichloroacetic acid filtrates of reaction mixtures were pipetted into 1 cm. spectrophotometer cells, and sufficient NaOH was added to yield a concentration of 1.0 M in a final volume of 3.0 ml. The optical density of these mixtures was read at 321 mμ in the Beckman spectrophotometer, and the content of phenylpyruvate calculated from the extinction coefficient of the product formed under these conditions ($\epsilon = 1.45 \times 10^7$ cm.^{2} per Mole phenylpyruvate). Nonspecific absorption of reaction mixtures was found to be negligible, but it

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TABLE I

*Effect of adult rat liver fractions on phenylalanine hydroxylase activity of liver preparations from fetal rats, rabbits, pigs and a human premature infant**

Experiment No.	Enzyme preparations added	μMoles tyrosine formed
1	Fetal rat liver soluble fraction	0.01
	Fetal rat liver soluble fraction + Fraction I	0.13
	Fetal rat liver soluble fraction + Fraction II	0.03
	Maternal rat liver soluble fraction	0.17
	Fractions I and II	0.07
2	Fetal rabbit liver soluble fraction	0.07
	Fetal rabbit liver soluble fraction + Fraction I	0.34
	Fetal rabbit liver soluble fraction + Fraction II	0.04
	Maternal rabbit liver soluble fraction	0.43
	Fractions I and II	1.76
3	Fetal pig liver soluble fraction	0.01
	Fetal pig liver soluble fraction + Fraction I	0.62
	Fetal pig liver soluble fraction + Fraction II	0.02
	Fractions I and II	0.07
4	Premature human infant liver soluble fraction	0
	Premature human infant liver soluble fraction + Fraction I	0.73
	Premature human infant liver soluble fraction + Fraction II	0
	Fractions I and II	0.98

* All assays were carried out for 60 minutes at 35° C. Enzyme preparations were added to beakers containing 150 μMoles phosphate buffer at pH 6.8, 2 μMoles reduced diphosphopyridine nucleotide, 2 μMoles L-phenylalanine, and sufficient 0.15 M KCl to yield a final volume of 3.0 ml. Soluble fractions were prepared from 33 per cent homogenates in 0.15 M KCl by centrifugation at 105,000 × G for 60 minutes; 1.0 ml. of this preparation was used in each experiment. In each experiment either of the adult rat liver fractions alone formed only traces (less than 0.01 μMole) of tyrosine; assay of the two together in the amounts used is included in the table. Rat fetuses were at term (22 days) and rabbit fetuses were at 21 days of gestation; in these experiments assays of preparations of the maternal liver are included for comparison.

was necessary to include controls for nonenzymic removal of phenylpyruvate, apparently due to binding to acid-precipitated protein. The optical densities of these mixtures decreased slowly with time and were read at a fixed interval, usually two minutes, after mixing.

Reagents. Diphosphopyridine nucleotide (DPN), triphosphopyridine nucleotide (TPN), sodium phenylpyruvate, and trishydroxymethylaminomethane (Tris) were all products of the Sigma Chemical Company. Reduced DPN (DPNH) was prepared by reaction of DPN with ethanol and alcohol dehydrogenase as described by Rafter and Colowick (19). Crystalline lactic and alcohol dehydrogenases were from the Worthington Biochemical Corporation. Glucose-6-phosphate dehydrogenase was prepared from dried yeast according to Kornberg and Horecker (20). The phenylalanine hydroxylase cofactor (2-amino-4-hydroxy-6,7-dimethyltetrahydropteridine) was a generous gift from Dr. Seymour Kaufman of the National Institutes of Health, Bethesda, Maryland.

RESULTS

Phenylalanine hydroxylation

It was first established by Mitoma (16) that the phenylalanine hydroxylase activity of rat liver can be dissociated by ammonium sulfate fractionation into two components. One of these (Fraction I) is found only in liver, is quite labile, and has been shown to be deficient in the livers of phenylketonuric individuals (21, 22). The second (Fraction II) is a stable enzyme which can be prepared from several tissues other than liver. Following the demonstration (11) that the livers of fetal and newborn rats are essentialy unable to form tyrosine from phenylalanine, experiments were carried out to determine whether this inactivity could be traced to deficiency in one, or in both, of the two enzymic components. Supplementing inactive soluble fractions of livers from fetal rats, rabbits and pigs with adult rat liver fractions shows quite conclusively that, in each case, the addition of Fraction I is sufficient to yield a combination with phenylalanine hydroxylase activity (Table I). A similar experiment was carried out using the soluble fraction of liver from a human premature infant, with the same result (Experiment 4, Table I). However, since this tissue was obtained at autopsy some time after the death of the infant, this result must be interpreted with caution, particularly in view of the apparent lability of this enzyme to autolysis (22).

Certain aspects of these experiments that were at first difficult to understand have been clarified by the studies of Kaufman (23, 24) which establish that this reaction requires a hitherto unrecognized cofactor. These aspects may be summarized as follows. *1*) While Fraction I loses activity rapidly on aging or dialysis when assayed with Fraction II, it was observed in several of these experiments that Fraction I preparations which had apparently lost most or all of their activity (when assayed with II) were still highly active in stimulating liver preparations from fetal animals (Experiments 1 and 3, Table I). *2*) As shown in Mitoma's initial report (16) and repeated in this laboratory, assay of the two rat liver fractions, varying one while holding the other constant, reveals important differences between them (Figure 1). If Fraction I is held at a constant level, and II varied, a typical enzyme concentration curve is obtained, while if II is constant and I varied, activity varies exponentially rather than linearly with the amount of I added. This suggests that Fraction I is providing more than one component to the system. *3*) As described in a preliminary report (12), the addition of varying amounts of Fraction I to inactive preparations of fetal liver results in activity which is essentially linearly related to the amount of I added; the

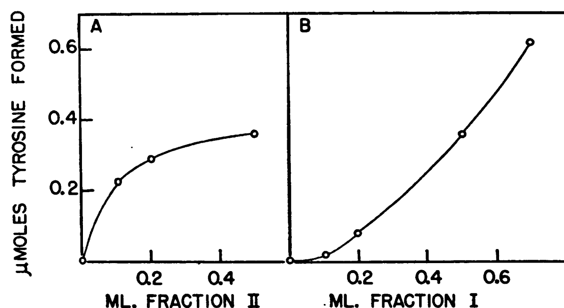

Fig. 1. Effect of Varying Amounts of Rat Liver Fractions in Hydroxylation of Phenylalanine

Reaction mixtures containing 150 μMoles phosphate buffer, pH 6.8, 4 μMoles L-phenylalanine, 1 μMole DPN, 10 μMoles sodium lactate, excess lactic dehydrogenase, rat liver fractions and 0.15 M KCl to 2.0 ml. were shaken at 35° C. for 30 minutes. After incubation trichloroacetic acid was added to five per cent, the mixtures were centrifuged and aliquots taken for tyrosine assay as described in "Methods." In A, all samples contained 0.5 ml. of Fraction I, and Fraction II was varied as indicated; in B, all contained 0.5 ml. of II, and I was varied as indicated.

TABLE II

*Specific requirement for hydroxylase (protein fraction I) by liver of fetal rats**

Additions	μMoles tyrosine formed
Fraction I	0
Fraction II	0.05
Fractions I and II	0.16
Fractions I and II + cofactor	0.91
Fetal rat liver soluble fraction	0.02
Fetal rat liver soluble fraction + Fraction I	0.27
Fetal rat liver soluble fraction + Fraction II	0.06
Fetal rat liver soluble fraction + cofactor	0

* The reaction mixtures contained 100 μMoles phosphate buffer at pH 6.8, 2 μMoles phenylalanine, 10 μMoles glucose-6-phosphate, 0.5 μMole triphosphopyridine nucleotide, 2 μMoles reduced diphosphopyridine nucleotide, excess yeast glucose-6-phosphate dehydrogenase, enzyme and cofactor additions as noted, and water to 1.5 ml. After 40 minutes at 37° C., trichloroacetic acid was added and the mixtures assayed for tyrosine as described in "Methods." The preparation of fetal rat liver was the 105,000 × G supernatant of a 33 per cent homogenate in 0.15 M KCl; 1.0 ml. of this preparation was used. Fractions I and II of adult liver were added in volumes containing 2.2 and 3.2 mg. of protein, respectively. Seventy-five μg. of the cofactor (2-amino-4-hydroxy-6, 7-dimethyltetrahydropteridine) was added where indicated.

multiple order response obtained when Fraction I is varied against II is not observed. These considerations can now be interpreted as indicating that Mitoma's Fraction I provides the rat liver system with the cofactor as well as with the specific hydroxylation enzyme, and that only one of these is required by fetal tissues. Thus the effect of Fraction I in stimulating inactive preparations from fetal animals could be due to deficiency of the fetal tissue in either of these components.

A direct test of this problem was made possible by Kaufman's discovery that the reduced form of several pteridine derivatives can effectively substitute for the natural cofactor, although none of those yet tested is identical with it (25). Among the most active is 2-amino-4-hydroxy-6,7-dimethyltetrahydropteridine, a sample of which was kindly made available to us by Dr. Kaufman. This cofactor was tested, along with aged protein fractions prepared from adult rat livers several days previously, for ability to stimulate hydroxylase activity of fetal rat liver. The results are summarized in Table II. It can be seen that this preparation of Fraction II is not completely free of I activity, and that I and II combined are relatively inactive unless supplemented with cofactor. This can be interpreted as indicating that I has

TABLE III

*Requirements for metabolism of phenylpyruvate by rat liver preparations**

Additions	μMoles phenylpyruvate metabolized
Homogenate	1.31
Mitochondria	0.21
Supernatant	0.27
Mitochondria + supernatant	1.18
Mitochondria + heated supernatant	1.22
Heated mitochondria + supernatant	0.15
Mitochondria + 5 μMoles L-glutamate	1.29

* Reaction mixtures containing 5 μMoles phenylpyruvate, 40 μMoles Tris buffer at pH 7.5, indicated additions and 0.25 M sucrose to 2.0 ml. were shaken at 37° C. for 30 minutes in the Dubnoff metabolic shaker, after which trichloroacetic acid was added and assays for phenylpyruvate carried out as described in "Methods." The mitochondria were washed three times with 0.25 M sucrose and the washings discarded; the supernatant fraction referred to is that part of the liver homogenate that is not sedimented at 8,500 × G. The amounts of each fraction added, in terms of equivalent weight of liver, were 0.5, 0.5 and 1.0 Gm. for homogenate, mitochondria and supernatant, respectively.

lost activity since its preparation, due to loss of the cofactor. Addition of II to the essentially inactive preparation from fetal rats stimulates to an extent attributable to the activity of II alone. Addition of the cofactor is without effect, while addition of I yields an active combination of even greater activity than I and II combined. This result establishes, then, that the fetal liver preparation contains both the cofactor and the enzyme of Fraction II, and is specifically deficient in the hydroxylase enzyme, represented by Fraction I.

Phenylalanine transamination

In preliminary studies of the routes of metabolism of phenylpyruvate, concentrated homogenates of rat liver were observed to metabolize this compound rapidly, either aerobically or anaerobically. On fractionation of homogenates by differential centrifugation, removal of phenylpyruvate could be shown to require both the mitochondria and the mitochondrial supernatant for maximal activity (Table III); the nuclear fraction (sedimented at 600 × G) was inactive. While the mitochondrial component is heat labile, the supernatant fraction lost no activity after boiling for five minutes and could be replaced by a variety of amino acids, of which glutamate was among the most active. These experiments could be repeated in an atmosphere of nitrogen without changing the results obtained.

The tentative conclusion that transamination was occurring was confirmed by paper chromatography of reaction mixtures; strong ninhydrin-positive spots corresponding to phenylalanine were obtained from each sample in which substantial amounts of phenylpyruvate had disappeared. A quantitative evaluation, measuring phenyalanine by the bacterial decarboxylase method of Udenfriend (26) was carried out, using washed mitochondria and glutamate under the conditions described in Table III. In duplicate samples, 1.83 and 1.87 μMoles phenylpyruvate disappeared and 1.75 and 1.78 μMoles phenylalanine were formed; in view of the uncertainties inherent in this phenylalanine assay these values were accepted as satisfactory stoichiometry.

The relative efficacy of several amino acids as amino donors in this reaction was studied in isolated subcellular fractions. Nuclear and microsomal fractions were essentially inactive with all the amino acids tested. Activity in mitochondrial and soluble fractions varied with each amino acid, but the distribution of activity in these fractions and the relative effectiveness of the amino acids were quite reproducible in several experiments. The data from a representative experiment are presented in Table IV. It is apparent that, when calculated in terms of an equivalent weight of

TABLE IV

*Effectiveness of various amino acids in transamination with phenylpyruvate in rat liver fractions**

Amino donor added	Phenylpyruvate disappeared (μMoles/hr./Gm. liver) Mitochondria	Soluble	Total
None	0.36	0.12	0.48
Glutamate	6.24	2.53	8.77
Alanine	2.48	2.13	4.61
Aspartate	4.92	0.08	5.00
Glutamine	7.48	4.13	11.61
Leucine	3.12	1.13	4.25

* Reaction mixtures containing 50 μMoles Tris buffer at pH 7.5, 30 μg. pyridoxal phosphate, 5 μMoles phenylpyruvate, 10 μMoles of the L-isomer of each amino acid indicated, tissue preparation and 0.25 M sucrose to 2.0 ml. were shaken for 60 minutes at 37° C. under N_2. Mitochondria, washed three times, were added in an amount equivalent to 0.5 Gm. of liver, while the amount of soluble fraction (105,000 × G supernatant) added was equivalent to 0.3 Gm. of liver. Assays for phenylpyruvate were carried out after acid addition as described in "Methods."

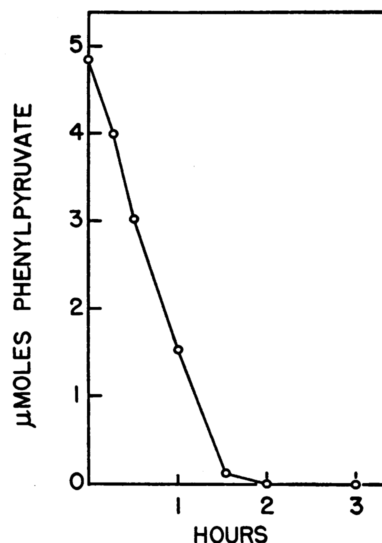

FIG. 2. TRANSAMINATION OF PHENYLPYRUVATE WITH L-GLUTAMINE IN RAT LIVER MITOCHONDRIA

The reaction mixture, containing 50 µMoles Tris buffer, pH 7.5, 60 µg. pyridoxal phosphate, 10 µMoles L-glutamine, 5 µMoles phenylpyruvate (4.84 µMoles by assay), three times washed mitochondria from 1 Gm. liver, and 0.25 M sucrose to 2.0 ml. was shaken at 37° C. under N_2 in the Dubnoff metabolic incubator. Small aliquots were taken at the times indicated and assayed for phenylpyruvate as described in "Methods."

liver, activity is rather evenly distributed between the two fractions. An exception is reaction with aspartic acid, which was in each experiment restricted to the mitochondria. These results suggest that there are probably several or many different transaminases capable of reaction with phenylpyruvate, although the possibility that some amino acids react indirectly, by prior formation of a specifically required donor, cannot be excluded. The rate of transamination with glutamate or glutamine is two to three times greater than with the other amino acids tested. Transamination with glutamine may be more rapid because this reaction goes to completion (Figure 2), probably due to coupling of transamination with deamidation of the α-ketoglutaramide formed (27). In contrast, reaction with glutamate tends toward equilibrium (Figure 3), as shown previously by Rowsell (9). It is of interest to note that the relative rates of this reversible reaction under the conditions used here (phenylalanine and phenylpyruvate at about 0.005 M, α-ketoglutarate and glutamate at 0.01 M) are quite different, the initial (first 30 minutes) rate in the direction of phenylalanine formation being approximately seven times greater than that of the reverse reaction. Rowsell found the two reactions to proceed at nearly equal rates, with all components added at 0.025 M. This disparity suggests a large difference in the Michaelis constant for the various reactants, with the phenylpyruvate-glutamate pair being bound much more tightly than phenylalanine and α-ketoglutarate.

Developmental study

The changes in activity of liver phenylpyruvate-glutamate transaminase that occur during late fetal and neonatal life in the rat are illustrated in Figure 4. Since this activity was shown to be distributed between mitochondrial and soluble fractions, the entire liver homogenates were assayed in this study. Under the conditions described in

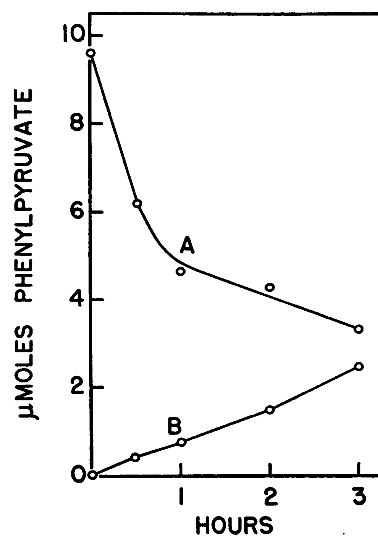

FIG. 3. APPROACH TO EQUILIBRIUM OF THE REVERSIBLE TRANSAMINATION BETWEEN PHENYLPYRUVATE AND L-GLUTAMATE IN RAT LIVER MITOCHONDRIA

Both reaction mixtures contained 50 µMoles Tris buffer, pH 7.5, 60 µg. pyridoxal phosphate, three times washed mitochondria from 1 Gm. liver, and sufficient 0.25 M sucrose to yield a final volume of 2.0 ml. In addition, 20 µMoles L-glutamate and 10 µMoles phenylpyruvate (9.6 µMoles by assay) were added to A, and 20 µMoles α-ketoglutarate and 10 µMoles L-phenylalanine were added to B. The mixtures were shaken at 37° C. under N_2; at the times indicated, appropriate aliquots were taken and assayed for phenylpyruvate as described in "Methods."

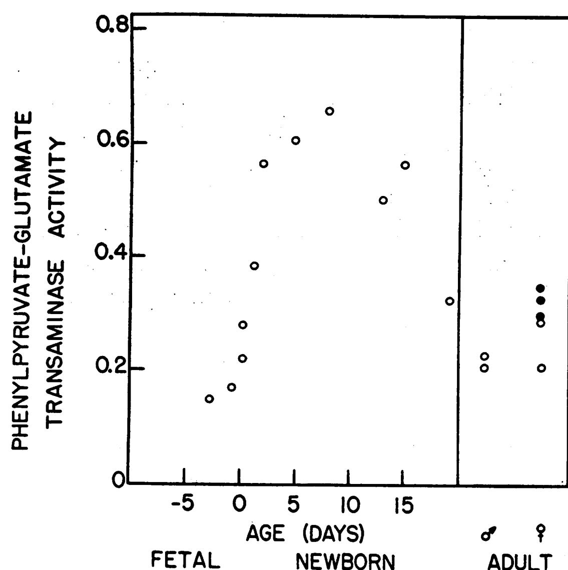

FIG. 4. CHANGES IN ACTIVITY OF HEPATIC PHENYLPYRUVATE-GLUTAMATE TRANSAMINASE DURING DEVELOPMENT OF THE RAT

Liver homogenates were shaken under N_2 for 30 minutes at 37° C. with 50 μMoles Tris buffer pH 7.5, 60 μg. pyridoxal phosphate, 10 μMoles phenylpyruvate and 20 μMoles L-glutamate in a total volume of 2.0 ml. After acid addition aliquots were assayed for phenylpyruvate as described in "Methods"; activity is expressed in terms of μMoles phenylpyruvate disappearing per hour per milligram homogenate protein. Adults are here considered as rats weighing 100 Gm. or more. The solid circles are representative of the activity of livers of gravid females.

Figure 4 the rate of transamination is roughly linear with homogenate concentration over a 10-fold range, and all assays were carried out in this range. The choice of glutamate as amino donor was due to the observation that of the amino acids tested, it, together with glutamine, was consistently the most active. Glutamine was not used because the rate of reaction with this donor is apparently dependent on deamidation as well as transamination. In several of these experiments reaction mixtures were assayed for phenylalanine by the bacterial decarboxylase procedure, and in each case the amount of phenylalanine formed agreed to within 10 per cent of that of phenylpyruvate that had been metabolized.

Activity is present in the terminal days of fetal life, and by the time of birth is equivalent to that found in the livers of adult rats. During the neonatal period activity is high, reaching a peak during the first 10 days, which is approximately twice the usual adult level. By the end of the third week activity has dropped to the adult level, and, although not shown, the activity of livers of animals older than three weeks was consistently within this range (0.20 to 0.35 μMole per hour per mg. protein). Little difference was noted between male and female adults and the slightly increased activity in livers of gravid females is probably not significant.

While this manuscript was in preparation a report was published describing changes in the activity of several enzymes of rat liver during development (28). Among the enzymes studied was phenylalanine-α-ketoglutarate transaminase, which was assayed in soluble fractions by following the tautomerization of phenylpyruvate formed. While the findings reported differ in some details from those described here, the overall pattern of change, including development during late fetal life and high activity during the neonatal period, was similar to that seen in Figure 4.

DISCUSSION

Perhaps the most notable feature of the patterns of development of the two enzyme systems that are considered here is their lack of uniformity. Activity of phenylalanine hydroxylase has been shown to be deficient in late gestation, as well as for the first day or two of postnatal life in the rat (11). Although the complex kinetics involved in the study of this enzyme system make an accurate evaluation of the activity of crude preparations quite difficult, it was shown that activity is similar to that of adults by the third to twelfth day after birth. In contrast, the capacity to form phenylpyruvate from phenylalanine by transamination is present during late fetal life, and is equivalent to that of adults by the time of birth. A limited series of analyses in this laboratory has indicated that the free phenylalanine content of the livers of fetal rats of 17 to 21 days of gestation is at least as high as that of adults, when measured by the bacterial decarboxylase procedure in perchloric acid filtrates of homogenates. The methods used in these studies are of necessity crude, and can yield no information concerning what may be important intracellular or intercompartmental shifts of metabolite concentration.

Thus, while substrate induction may be involved in the mechanism of enzyme formation, these findings indicate that it is not simply the amount of substrate available that determines the time course of enzyme development. In this connection it might be noted that the development of tyrosine-α-ketoglutarate transaminase, an enzyme metabolically related to those considered here, has been shown to be under a measure of hormonal control (13).

The results reported here also establish that the characteristic inability of the livers of fetal mammals to form tyrosine from phenylalanine is due to absence or inactivity of the specific hydroxylase enzyme, as both the nonspecific enzyme and the cofactor are present. A recent report by Kaufman (25) has shown that biopsy specimens of liver from phenylketonuric individuals contain the cofactor as well as the nonspecific enzymic component of the hydroxylase system. Further study of the factors controlling the development of this enzyme in the newborn animal may prove to be of significance in this regard.

SUMMARY

Liver preparations from fetal rats, rabbits, pigs and a human premature infant were found to be essentially incapable of forming tyrosine from phenylalanine. Supplementing these inactive preparations with adult rat liver fractions indicates that each is deficient in the specific component of the phenylalanine hydroxylase system. The activity of fetal rat liver is not affected by addition of 2-amino-4-hydroxy-6,7-dimethyltetrahydropteridine, which is active as a cofactor in this system, indicating that inactivity is due to deficiency of the hydroxylase enzyme.

Phenylpyruvate undergoes transamination with several amino donors in the mitochondrial and soluble fractions of rat liver; glutamate and glutamine are particularly effective. In mitochondria, reaction with glutamine goes to completion, while reaction with glutamate approaches equilibrium. Assay of phenylpyruvate-glutamate transamination in the livers of fetal and neonatal rats indicates that activity is present during late fetal life, reaches the adult level by birth, and is higher than that of the adult during the neonatal period. Differences in the developmental patterns of phenylalanine hydroxylation and transamination are discussed from the point of view of substrate control.

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REFERENCES

1. Cohn, M. Contributions of studies on the β-galactosidase of *Escherichia coli* to our understanding of enzyme synthesis. Bact. Rev. 1957, **21**, 140.
2. Knox, W. E., and Mehler, A. H. The adaptive increase of the tryptophanperoxidase-oxidase system of the liver. Science 1951, **113**, 237.
3. Lin, E. C. C., and Knox, W. E. Adaptation of the rat liver tyrosine-α-ketoglutarate transaminase. Biochim. biophys. Acta 1957, **26**, 85.
4. Gordon, M. W., and Roder, M. Adaptive enzyme formation in the chick embryo. J. biol. Chem. 1953, **200**, 859.
5. Kato, Y., and Moog, F. Difference in response of phosphatases in chick embryo to injection of substrate. Science 1958, **127**, 812.
6. Spiegelman, S. Differentiation as the controlled production of unique enzymatic patterns. Symp. Soc. exp. Biol. 1948, **2**, 286.
7. Meister, A., and Tice, S. V. Transamination from glutamine to α-keto acids. J. biol. Chem. 1950, **187**, 173.
8. Rowsell, E. V. Transaminations with pyruvate and other α-keto acids. Biochem. J. 1956, **64**, 246.
9. Rowsell, E. V. Transaminations with L-glutamate and α-oxoglutarate in fresh extracts of animal tissues. Biochem. J. 1956, **64**, 235.
10. Udenfriend, S., and Cooper, J. R. The enzymatic conversion of phenylalanine to tyrosine. J. biol. Chem. 1952, **194**, 503.
11. Reem, G. H., and Kretchmer, N. Development of phenylalanine hydroxylase in liver of the rat. Proc. Soc. exp. Biol. (N. Y.) 1957, **96**, 458.
12. Kenney, F. T., Reem, G. H., and Kretchmer, N. Development of phenylalanine hydroxylase in mammalian liver. Science 1958, **127**, 86.
13. Sereni, F., Kenney, F. T., and Kretchmer, N. Factors influencing the development of tyrosine-α-ketoglutarate transaminase activity in rat liver. J. biol. Chem. 1959, **234**, 609.
14. Schneider, W. C. Intracellular distribution of enzymes. III. The oxdiation of octanoic acid by rat liver fractions. J. biol. Chem. 1948, **176**, 259.
15. Kenney, F. T., Colowick, S. P., and Barbehenn, E. The formation of inorganic pyrophosphate in microsomes. Arch. Biochem. 1957, **69**, 617.
16. Mitoma, C. Studies on partially purified phenylalanine hydroxylase. Arch. Biochem. 1956, **60**, 476.

17. Udenfriend, S., and Cooper, J. R. The chemical estimation of tyrosine and tyramine. J. biol. Chem. 1952, **196**, 227.
18. Gilvarg, C. Prephenic acid and the aromatization step in the synthesis of phenylalanine *in* A Symposium on Amino Acid Metabolism, W. D. McElroy and B. Glass, Eds. Baltimore, Johns Hopkins Press, 1955, p. 812.
19. Rafter, G. W., and Colowick, S. P. Enzymatic preparation of DPNH and TPNH *in* Methods in Enzymology, S. P. Colowick and N. O. Kaplan, Eds. New York, Academic Press Inc., 1957, vol. III, p. 887.
20. Kornberg, A., and Horecker, B. L. Glucose-6-phosphate dehydrogenase *in* Methods in Enzymology, S. P. Colowick and N. O. Kaplan, Eds. New York, Academic Press Inc., 1955, vol. I, p. 323.
21. Wallace, H. W., Moldave, K., and Meister, A. Studies on conversion of phenylalanine to tyrosine in phenylpyruvic oligophrenia. Proc. Soc. exp. Biol. (N. Y.) 1957, **94**, 632.
22. Mitoma, C., Auld, R. M., and Udenfriend, S. On the nature of enzymatic defect in phenylpyruvic oligophrenia. Proc. Soc. exp. Biol. (N. Y.) 1957, **94**, 634.
23. Kaufman, S. The enzymatic conversion of phenylalanine to tyrosine. Biochim. biophys. Acta 1957, **23**, 445.
24. Kaufman, S. Enzymatic conversion of phenylalanine to tyrosine. Fed. Proc. 1957, **16**, 203.
25. Kaufman, S. Phenylalanine hydroxylation cofactor in phenylketonuria. Science 1958, **128**, 1506.
26. Udenfriend, S. Assay of aromatic amino acids *in* Methods in Enzymology, S. P. Colowick and N. O. Kaplan, Eds. New York, Academic Press Inc., 1957, vol. III, p. 607.
27. Meister, A. Transamination. Advanc. Enzymol. 1955, **16**, 185.
28. Auerbach, V. H., and Waisman, H. A. Tryptophane peroxidase-oxidase, histidase, and transaminase activity in the liver of the developing rat. J. biol. Chem. 1959, **234**, 304.